Q-341

Fecal Source Tracking in a Storm Drain System Using Multiple Sewage Indicators in Conjunction with *Enterococcus* Real-Time qPCR Enumeration and Speciation

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INTRODUCTION

Fecal pollution, characterized by elevated concentrations of fecal indicator bacteria such as *Escherichia coli* and *Enterococcus* spp. in the water column and increased risk of exposure to microbial pathogens, is one of the leading causes of water quality impairment in New England recreational surface waters. Finding and correcting chronic and episodic fecal pollution of surface waters is an important public health and environmental goal for the U.S. EPA and state environmental regulatory agencies. Quincy Bay, Quincy, MA, is a relatively shallow bay with a 7-day water residence time. Wollaston Beach, a Massachusetts Department of Conservation & Recreation (DCR) marine beach in Quincy Bay, is heavily impacted by storm water from 8 outfalls running the length of the beach. These outfalls drain 8 basins, the largest of which (Outfall # 6) extends far west to the adjacent town line (see Fig. 2) along the basic drainage path of Sachem Brook. During storm events exceeding ½ inch of rain in a 24-h period, Wollaston Beach is generally closed to swimming due to elevated enterococci concentrations. There is an urgent public health need for faster enterococci analytical methods to test recreational waters and offer timely warning to the bathing public as well as for accurate analytical methods to help identify and eliminate illicit sources of sewage contamination in recreational waters.

OBJECTIVES

- To evaluate multiple fecal source tracking methods using samples taken from Outfall # 6 at its discharge point in Wollaston Beach and at selected upstream points in the storm drain system.
- To evaluate rapid *Enterococcus* enumeration and speciation methods using samples taken from the Outfall # 6 storm drain system.
- To assess the fecal source(s) in the Outfall # 6 storm drain system impacting Wollaston Beach.

METHODOLOGY

- Eight rounds of sampling for enterococci enumeration were conducted on the Outfall # 6 storm drain system from April through September 2007 (see Table 1, Fig. 2, and Sampling Stations Map).
- Enterococci were enumerated in storm drain water samples using a real-time quantitative PCR (qPCR) method [EPA Method 1606 – $\log_{10}$ AQM (N)/100 mL, $\log_{10}$ SPEC Equivalent, and $\log_{10}$ CFU Equivalent] and two cultural methods – EPA Method 1600 ($\log_{10}$ CFU/100 mL) and Enterolert™ - Quanti-Tray®-2000† (IDEXX, Westbrook, ME – $\log_{10}$ MPN/100 mL).
- Samples from two sampling rounds (May 21 and September 19, 2007) were also tested for *Enterococcus* species composition using capillary electrophoresis of the *groESL* gene spacer region and for the following sewage-specific bacterial DNA markers and anthropogenic chemicals:
  - Fecal *Bacteroidetes* human markers (HF134 and HF183) using a PCR assay.
  - *Enterococcus faecium* excellular surface protein (*esp*) marker using a PCR assay.
  - Fluorescent whitening agents (FWAs) by solid-phase extraction followed by HPLC analysis.
  - Caffeine by solid-phase extraction followed by GC/MS analysis.
- Wollaston Beach enterococci monitoring data for beach water samples and rainfall data were obtained from the MWRA & Blue Hill Observatory websites, respectively.

REFERENCES

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ACKNOWLEDGEMENTS

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- We also gratefully acknowledge Philip Murphy of MassGIS for cartographic assistance.

† Use of trade or firm names is for identification purposes only and does not constitute endorsement by MassDEP or the U.S. EPA.

EXPERIMENTAL DATA

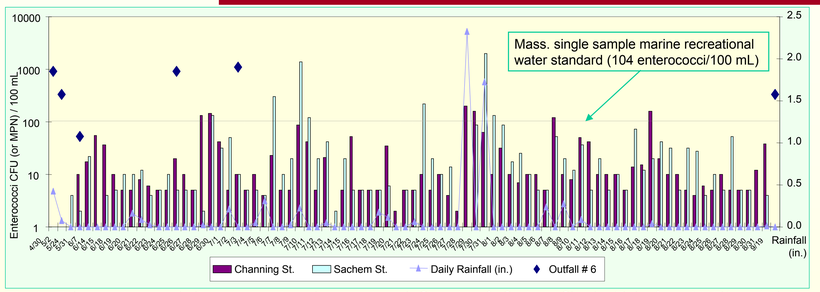


Fig. 1. Enterococci concentrations in beach water samples at Channing & Sachem Streets (Data from MWRA website – http://www.mwra.state.ma.us/harbor/html/beachdata.htm) and in samples from Outfall # 6 discharge point versus daily rainfall data from April through September 2007.

Fig. 2. Map of eight storm outfall drainage systems located along Wollaston Beach and location of MWRA beach water sampling sites and sampling stations along Outfall # 6.

Table 1. Wollaston Beach MST project sample locations, field cross ID, and references.

Station #	Location	Dates Sampled / Original Designations						
		4/23/07	4/25/07	4/30/07	5/2/07	5/21/07	6/15/07	7/2/07	9/19/07
0	SWO -05	000	ns	000	000	000	000	000	000
0-1	Quincy Shore Drive @ Beach St.	002	ns	ns	000-2	ns	000-1	001-1	ns
0-2	Quincy Shore Drive @ Vassal St.	ns	ns	ns	ns	ns	000-2	001-2	ns
01	Quincy Shore Drive @ Ocean Cove Appartment Parking Lot	ns	ns	001	001	001	001	001	001
02	Billings Rd. Upstream of Old Tide Gate	ns	011	002	002	002	002	002	003
03	Cummings Ave.	002	012	003	003	003	003	003	002
04	Willet St. @ Oxenbridge Rd.	004	014	004	004	004	004	004	00
05	Willet St. @ Hancock St.	005	015	005	005	005	005	005	005 & 005D 005D is FD of 005
05-1	Woodbine St. @ Greenwood Ave.	ns	ns	ns	ns	012	006-1	006-1 & 005 005 is FD of 006-1	005-1
05-2	Woodbine St. @ MBTA Station	ns	ns	ns	ns	ns	ns	006-2	ns
06	Newport Ave. @ Brook St.	006	016	006	006	006	006	006	006
07	Brook St. @ Farrington St.	007	017	007	007	ns	009	ns	ns
08	Brook St. @ Taylor St.	ns	ns	ns	ns	007	ns	ns	007
09	Brook St. @ Highland Ave.	008	ns	008	008	ns	ns	ns	ns
10	Brook St. @ North Central	009	ns	009	ns	ns	ns	ns	ns
11	South Central @ Beale St.	ns	018	010	ns	008	ns	009	ns
12	Sewer @ Brook St. @ Taylor St.	ns	ns	ns	010	013	ns	ns	013
BLK	Trip Blank	ns	ns	ns	010	011	intial/final	0	011

Table 2. Enterococci concentrations in storm drain water samples (Outfall # 6) determined by qPCR and Enterolert™ (mean ± SD)‡

Station #	Distance to Outfall (ft)	# of Samples	Log AQM (N / 100mL)	Log SPEC Equivalent	Log CFU Equivalent	Log MPN/100mL
000	0	6	4.91 ± 0.75^{ab}	3.79 ± 0.70^{ab}	3.66 ± 0.70^{ab}	2.62 ± 0.50^{def}
000-1	290	3	4.13 ± 0.46^{a}	2.93 ± 0.50^{a}	2.80 ± 0.50^{a}	2.16 ± 1.16^{bcde}
000-2	310	2	6.47 ± 1.42^{c}	5.43 ± 1.67^{c}	5.31 ± 1.67^{c}	3.38 ± 0.00^{f}
001	320	6	4.93 ± 0.39^{ab}	3.71 ± 0.38^{ab}	3.59 ± 0.38^{ab}	2.37 ± 0.76^{cdef}
002	1000	8	5.33 ± 0.72^{b}	4.20 ± 0.79^{b}	4.08 ± 0.79^{b}	1.90 ± 0.80^{bcd}
003	1670	8	5.35 ± 0.37^{b}	4.20 ± 0.31^{b}	4.07 ± 0.31^{b}	1.74 ± 0.56^{bcd}
004	3220	8	6.62 ± 0.67^{c}	5.51 ± 0.64^{c}	5.41 ± 0.61^{c}	2.97 ± 0.57^{def}
005	7190	8	7.12 ± 0.42^{c}	5.91 ± 0.42^{c}	5.82 ± 0.44^{c}	3.15 ± 0.25^{ef}
005-1	7790	4	4.39 ± 0.36^{ab}	3.26 ± 0.32^{ab}	3.13 ± 0.32^{ab}	1.85 ± 0.80^{bcd}
005-2	8090	2	4.60 ± 0.53^{ab}	3.54 ± 0.53^{ab}	3.42 ± 0.53^{ab}	1.24 ± 0.14^{abc}
006	8390	8	4.23 ± 0.33^{a}	3.08 ± 0.33^{a}	2.96 ± 0.34^{a}	0.95 ± 0.88^{ab}
007	9055	4	4.34 ± 0.53^{ab}	3.12 ± 0.53^{a}	2.99 ± 0.53^{a}	0.46 ± 0.74^{a}
008	9575	2	4.71 ± 1.18^{ab}	3.60 ± 1.23^{ab}	3.48 ± 1.23^{ab}	1.24 ± 0.91^{abc}
009	9835	3	4.27 ± 0.49^{a}	3.05 ± 0.49^{a}	2.93 ± 0.49^{a}	2.09 ± 0.21^{bcde}
010	10265	4	3.94 ± 0.75^{a}	2.72 ± 0.76^{a}	2.60 ± 0.76^{a}	2.43 ± 1.18^{cdef}
011	10925	5	4.97 ± 1.10^{ab}	3.80 ± 1.13^{ab}	3.68 ± 1.13^{ab}	2.15 ± 1.22^{bcde}
All Stations		81	5.19±1.13	4.03±1.14	3.91±1.15	2.10±1.02

‡Statistical significance by sampling station grouped using Duncan's Test at $p \leq 0.05$

Table 3. Kendall *tau* correlations for enterococci enumeration data & other results.

	Log AQM N/100mL	Log SPEC Equivalent	Log CFU Equivalent	Log CFU /100mL	Log MPN /100mL	*E. faecium esp* gene	*Bacteroidetes* Human Marker	FWAs	Caffeine	*E. faecalis* (%)
Log AQM (N/ 100mL)	---									
Log SPEC Equivalent	0.918***	---								
Log CFU Equivalent	0.930***	0.988***	---							
Log CFU/100mL	0.653***	0.641***	0.629***	---						
Log MPN/100mL	0.637***	0.602***	0.591***	0.759***	---					
E. faecium esp gene	0.161	0.145	0.161	0.024	0.113	---				
Bacteroidetes Human Marker	0.375*	0.359*	0.359*	0.287	0.326	0.169	---			
FWAs	0.592***	0.592***	0.592***	0.525**	0.552**	-0.055	0.240	---		
Caffeine	0.235	0.275	0.262	0.196	0.047	-0.130	0.331	0.433**	---	
Enterococcus faecalis (%)	0.650***	0.633***	0.617***	0.577**	0.617***	-0.127	0.306	0.532**	0.237	---

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

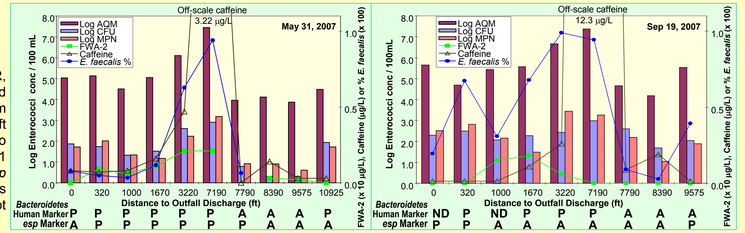
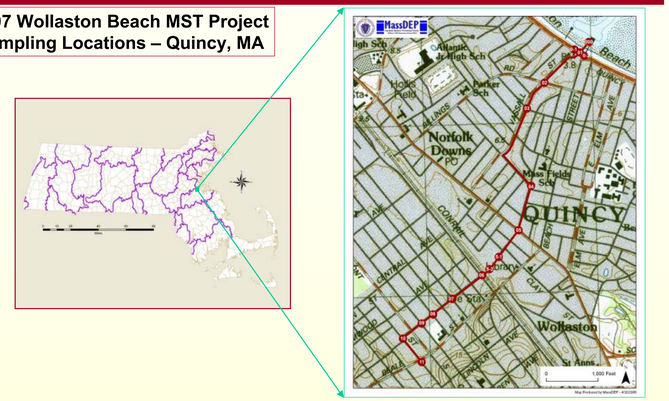


Fig. 3. Concentration of enterococci ($\log_{10}$ - measured by qPCR, Enterolert™, and EPA Method 1600), FWA #2, and caffeine, and % *E. faecalis* in samples taken along a storm drain system (Outfall # 6) on two dates – May 31, 2007 (left panel) and September 19, 2007 (right panel). Note that no samples were collected for FWA testing from Station 005-1 (7790 ft) on May 31, 2007. Results for the *E. faecium esp* sewage marker and fecal *Bacteroidetes* human markers are shown as P (present), A (absent), or ND (not determinable due to PCR inhibition).

SAMPLING STATIONS

2007 Wollaston Beach MST Project Sampling Locations – Quincy, MA



RESULTS & DISCUSSION

- Enterococci concentrations from qPCR and Enterolert™ analysis of eight rounds of samples collected from April through September 2007 from Outfall # 6 at its discharge point in Wollaston Beach and at upstream storm drain locations are shown in Table 2. Analysis of this data set using Student's paired *t* test demonstrated that the enterococci enumeration results from the qPCR assay ($\log_{10}$ CFU Equivalent) were significantly higher ($p < 0.001$; n = 81) than those from the Enterolert™ Test. Enterococci qPCR results were also statistically higher ($p \leq 0.05$) for samples taken from stations 310, 3220, and 7190 ft upstream of Outfall # 6 (see Table 2).
- Enterococci results (by Enterolert™) for samples from Outfall # 6 at its discharge point in Wollaston Beach are plotted in Fig. 1 along with enterococci beach water data (by EPA Method 1600) for two DCR beach sampling stations (i.e., off Channing St. and off Sachem St. – one on each side of Outfall # 6) and rainfall data.
  - Samples collected directly from Outfall # 6 had much higher enterococci concentrations than beach water samples from the two nearby DCR monitoring stations. Outfall # 6 was observed to be constantly flowing regardless of weather conditions. Beach water enterococci concentrations exceeded the Massachusetts standard for marine recreational water, primarily following wet weather.
- *Enterococcus* enumeration in storm drain water samples by qPCR and both cultural methods were significantly correlated with one another (Kendall *tau* correlation coefficients = 0.637 – 0.759, $p < 0.001$) (see Table 3).
- In storm drain water samples from two sampling rounds that were also tested for *Enterococcus* species composition, and for sewage-specific bacterial DNA markers and anthropogenic chemicals:
  - FWAs and % *E. faecalis* were significantly correlated with each other (Kendall *tau* = 0.552, $p < 0.01$) as well as with the enterococci qPCR (Kendall *tau* = 0.592 – 0.650, $p < 0.001$) and cultural results (Kendall *tau* = 0.525 – 0.617, $p < 0.01$) (Table 3).
  - FWAs and caffeine were also significantly correlated with each other (Kendall *tau* = 0.433, $p < 0.01$).
  - No significant correlations at $p < 0.01$ were found between the *Bacteroidetes* markers or *esp* marker and other parameters, but this evaluation may have been hampered by the non-quantitative nature of these DNA marker assays (Table 3).
  - Sewage contamination was traced to Station # 005, 7190 ft upstream of Outfall # 6, where high concentrations of enterococci (by qPCR and cultural methods), FWAs, and caffeine were found, along with the presence of both *Bacteroidetes* human markers (the *esp* marker was also detected in one sampling round) and species composition dominated (> 90%) by *E. faecalis*. (Fig. 3 & Table 2).
- The results of this study clearly show that rapid enumeration (qPCR) and speciation of enterococci coupled with multiple sewage-specific bacterial DNA markers and anthropogenic chemicals can be used to quickly and accurately track illicit sewage sources in storm drain systems.